J Formos Med Assoc 2011;110(2):93–99

Contents lists available at ScienceDirect

Journal of the Formosan Medical Association

Journal homepage: http://www.jfma-online.com

Original Article

# Differential Toxicities of Intraneurally Injected Mercuric Chloride for Sympathetic and Somatic Motor Fibers: An Ultrastructural Study

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**Background/Purpose:** Mercury is a well-known neurotoxin but the susceptibility of autonomic nerves to mercury poisoning *in vivo* has seldom been studied. Our previous studies have shown that the hypoglossal nerve in hamsters contains somatic motor and postganglionic sympathetic fibers. The aim of this study was to investigate the ultrastructural changes in the nervous system following intraneural injection of mercuric chloride into the hypoglossal nerve in hamsters.

**Methods:** Six adult hamsters were used in this study. After anesthesia, the digastric muscle on the right side was removed and the trunk of the hypoglossal nerve was exposed. Two microliters of mercuric chloride aqueous solution was injected into the main trunk of the hypoglossal nerve at the bifurcation. The contralateral hypoglossal nerve was kept intact and used as the normal control. Animals were allowed to survive for 1 or 3 days and were prepared for ammonium sulfide histochemistry and electron microscopy.

**Results:** Three days after injection of mercuric chloride solution, almost all unmyelinated sympathetic fibers in the hypoglossal nerve trunk were lost, whereas myelinated somatic axons were spared. Although mercury deposition in the myelin sheaths of neuronal processes was observed in the hypoglossal nucleus, the neuronal somas were intact. By contrast, degenerated neuronal processes and mercury deposition in neuronal somas were frequently found in the superior cervical ganglia.

**Conclusion:** This study demonstrated an undue susceptibility of sympathetic fibers to mercury intoxication. The mechanisms that underlie the selective reaction of sympathetic fibers to mercury warrant further investigation.

**Key Words:** hamster, mercuric chloride, neurotoxicity, sympathetic nerves, somatic motor nerves



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**Received:** January 7, 2010
**Revised:** January 18, 2010
**Accepted:** January 20, 2010

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Mercury is a well-recognized neurotoxin. However, mercury neurotoxicity presents in many ways, depending on the type of exposure and chemical form of the metal. The classic symptoms associated with exposure to elemental mercury vapor and methyl mercury involve the central nervous system, whereas inorganic mercury intoxication might produce peripheral neuropathy in addition to pathological changes in the kidney and gastrointestinal system.[1] Despite these differences, systemic toxicity experiments have demonstrated that organic and inorganic mercury compounds produce identical histological changes in the kidneys[2] and dorsal root ganglion neurons.[3] Furthermore, direct injections of the two classes of compounds into the rat cerebrum[4] and sciatic nerve[5] induce similar degeneration of nervous tissues. It has been suggested that the relative bioavailability in the nervous system, rather than any inherent neurotoxicity of the compounds, accounts for their different neurological effects.[6]

Another interesting issue in mercury neurotoxicity is the selective vulnerability of the nerve cells to the toxic agents. Systemic mercury intoxication in rats produces extensive degenerative changes in the neurons of the dorsal root ganglia and granule cells of the cerebellum, whereas moderate or no pathological lesions are found in the Purkinje cells and anterior horn motoneurons.[3,7] In the peripheral nerves, sensory fibers appear to be more sensitive to mercury toxicity and show more severe and extensive pathological changes than the motor fibers.[7,8] However, studies on the toxic effects of mercury on the autonomic nervous system have been relatively rare. Although systemic mercury intoxication produces alterations in autonomic functions,[9] and mercury induces toxic effects in sympathetic neurons *in vitro*,[10] the susceptibility of autonomic nerves to mercury toxicity *in vivo* has seldom been studied. Moreover, the differential effects of mercury on autonomic and somatic nerves are largely unknown.

Our previous studies have shown that the hypoglossal nerve in hamsters contains postganglionic sympathetic fibers in addition to the somatic motor component.[11] It provides a system that can be used to compare the effects of neurotoxins on sympathetic and somatic motor neurons. The aim of the present study was to investigate the ultrastructural changes in the nervous system following intraneural injection of mercuric chloride into the hypoglossal nerve in hamsters, with an emphasis on the differential reactions of sympathetic and somatic motor fibers to the neurotoxin.

## Materials and Methods

### *Mercuric chloride injection*

Six adult hamsters of both sexes weighing 120–150 g were used in this study. The experimental protocol was approved by the Center of Laboratory Animals, College of Medicine, National Taiwan University. The animals were maintained following the Guide to Management and Use of Experimental Animals, National Science Council, Taiwan. After anesthesia with intraperitoneal injection of 7% chloral hydrate (0.6 mL/100 g), the digastric muscle on the right side was removed and the trunk of the hypoglossal nerve was exposed using an aseptic surgical technique. The hypoglossal nerve bifurcates into medial and lateral branches at this level. Two microliters of mercuric chloride aqueous solution (75 mg/mL) was injected into the main trunk of the hypoglossal nerve at the bifurcation using a 5-μL Hamilton syringe in conjunction with a glass microneedle. To prevent leakage of mercuric chloride solution, a small amount of pure petroleum gel was applied over the injection site. The contralateral hypoglossal nerve was kept intact and used as the normal control. After the operation, the skin incision was closed and the animals were allowed to survive for 1 or 3 days (3 animals in each group).

### *Perfusion and fixation*

Following deep anesthesia, the animals underwent intracardiac perfusion with Ringer's solution, followed by an aldehyde mixture that contained 1.25% glutaraldehyde and 1% paraformaldehyde

in 0.1 M phosphate buffer at pH 7.4. After perfusion, the medulla, superior cervical ganglia, and the trunks of the hypoglossal nerve, 0.5 cm proximal to the injection site, were removed carefully under a surgical microscope. Tissue specimens were fixed in the same fixative for a further 2 hours.

### *Ammonium sulfide histochemistry*

For histochemical study, the specimen was embedded in agar and cut into 100-μm-thick sections on a vibratome. The sections were incubated in 0.5% aqueous solution of ammonium sulfide on a rotary shaker at room temperature for 15 minutes to form the immobile mercuric sulfide precipitant. To terminate the reaction, the sections were washed in 0.1 M phosphate buffer, pH 7.4, for 1 minute.

### *Electron microscopy*

The tissue sections were post-fixed with 2% osmic acid in phosphate buffer (0.1 M, pH 7.4) for 1 hour, dehydrated in a graded alcohol series, and embedded in Epon–Araldite mixture. Ultrathin sections were cut on a Reichert–Jung ultramicrotome and collected on 150-mesh grids. Without staining, the sections were examined under a JEOL 2000EX electron microscope.

## Results

### *Ultrastructural changes in hypoglossal nerve trunk after intraneural injection of mercuric chloride*

In the control nerves, large myelinated axons and small unmyelinated fibers grouped in bundles were clearly seen (Figure 1). One day after mercuric chloride injection, the number of unmyelinated axons decreased dramatically. Scattered unmyelinated fibers that showed axoplasmic vacuolation and signs of degeneration were occasionally found (Figure 2A). The myelinated fibers were largely preserved but mercuric sulfide particles were found in the myelin layers (Figure 2A). Three days after injection, disintegration of the myelin layer began

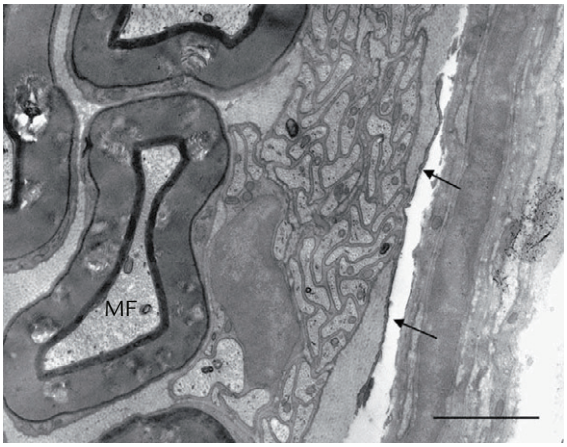


**Figure 1.** Transverse section of a control hypoglossal nerve showing a bundle of small unmyelinated fibers beneath the cytoplasmic lamella of an endoneurial fibrocyte (arrows) in the periphery of the nerve. Note the large-caliber myelinated fibers in the vicinity. Bar = 2.5 μm; MF = Myelinated fibers.

to appear (Figure 2B). Mercury deposition and lamellar bodies were seen in large myelinated axons (Figure 2B), and membrane-bound bodies that contained vacuoles and organelles were sometimes found in the axoplasm (Figure 2C). In the cytoplasm of related Schwann cells, vacuolation and disorganization of the organelles were obvious (Figure 2C). No unmyelinated fibers were identified in the hypoglossal nerve trunk 3 days after injection of mercuric chloride.

### *Ultrastructural changes in hypoglossal nucleus after intraneural injection of mercuric chloride*

One day after injection of mercuric chloride, electron-dense mercuric sulfide particles that were deposited in the myelin sheath began to appear in the hypoglossal nucleus, but the ultrastructure of the neuronal somas and processes was largely unchanged (Figure 3A). Three days after injection, myelin disorganization was sometimes found. Lipofuscin granules and multivesicular bodies that contained electron-dense mercuric sulfide particles were observed in the neuronal processes (Figure 3B), but the neuronal somas were largely intact (Figure 3C). No morphological changes were observed in the microvessels of the hypoglossal nucleus after intraneural injection of mercuric chloride into the hypoglossal nerve (Figure 3C).

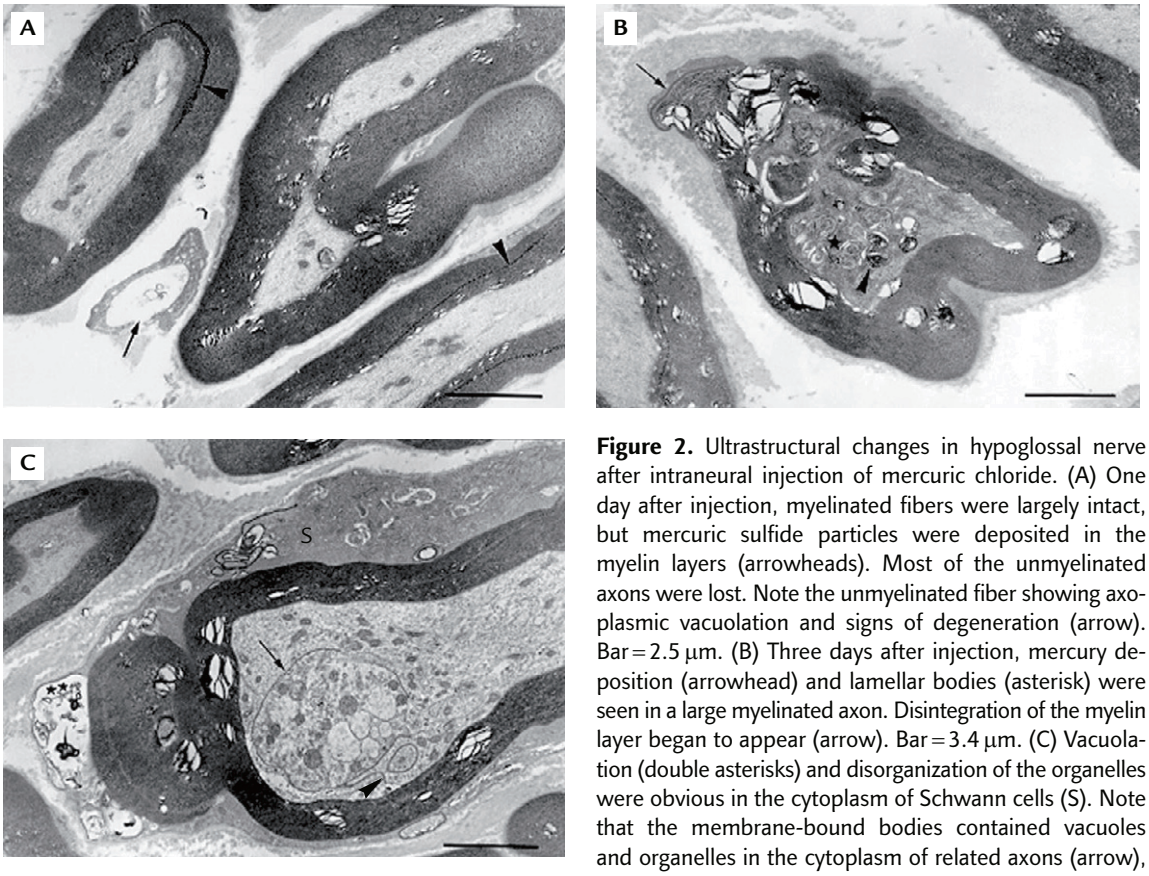


**Figure 2.** Ultrastructural changes in hypoglossal nerve after intraneural injection of mercuric chloride. (A) One day after injection, myelinated fibers were largely intact, but mercuric sulfide particles were deposited in the myelin layers (arrowheads). Most of the unmyelinated axons were lost. Note the unmyelinated fiber showing axoplasmic vacuolation and signs of degeneration (arrow). Bar = 2.5 μm. (B) Three days after injection, mercury deposition (arrowhead) and lamellar bodies (asterisk) were seen in a large myelinated axon. Disintegration of the myelin layer began to appear (arrow). Bar = 3.4 μm. (C) Vacuolation (double asterisks) and disorganization of the organelles were obvious in the cytoplasm of Schwann cells (S). Note that the membrane-bound bodies contained vacuoles and organelles in the cytoplasm of related axons (arrow), and the membranes were connected with the myelin sheaths (arrowhead). Bar = 5.0 μm.

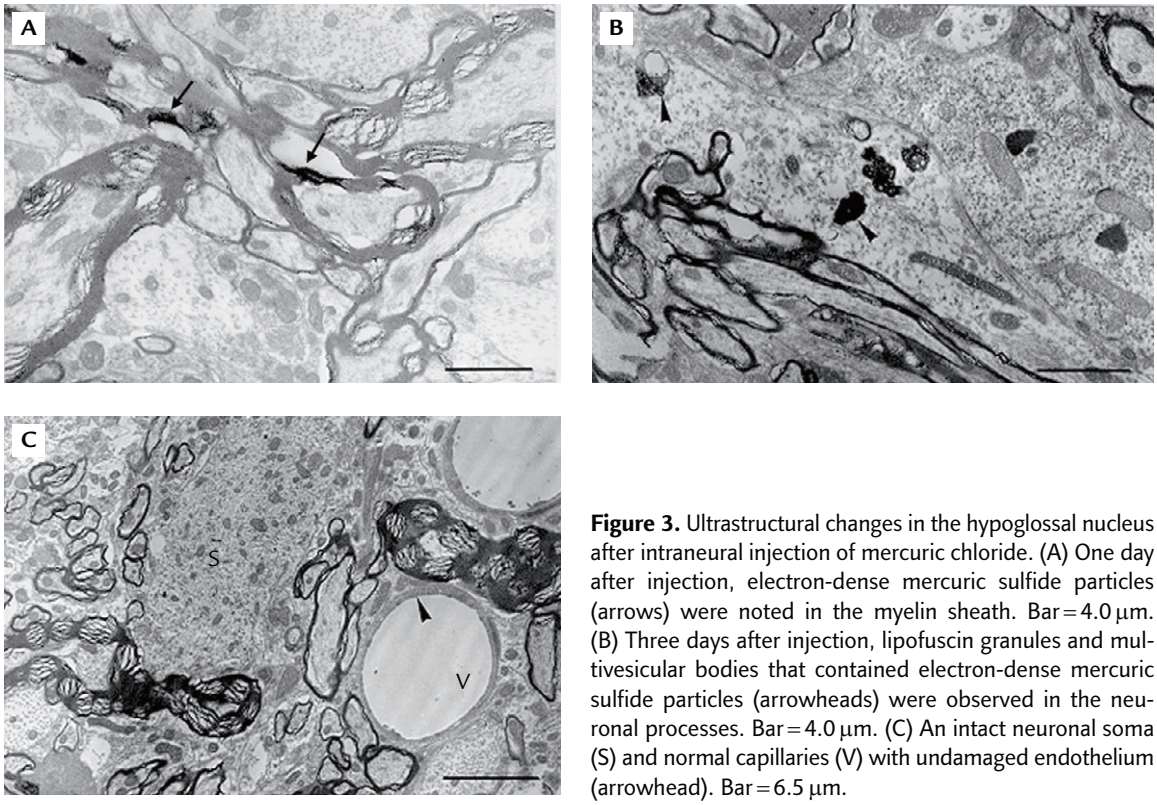


**Figure 3.** Ultrastructural changes in the hypoglossal nucleus after intraneural injection of mercuric chloride. (A) One day after injection, electron-dense mercuric sulfide particles (arrows) were noted in the myelin sheath. Bar = 4.0 μm. (B) Three days after injection, lipofuscin granules and multivesicular bodies that contained electron-dense mercuric sulfide particles (arrowheads) were observed in the neuronal processes. Bar = 4.0 μm. (C) An intact neuronal soma (S) and normal capillaries (V) with undamaged endothelium (arrowhead). Bar = 6.5 μm.

### *Ultrastructural effects of intraneurally injected mercuric chloride on superior cervical ganglion*

Ultrastructural changes in the superior cervical ganglion were similar at 1 day and 3 days after injection of mercuric chloride. After injection, degenerating neuronal processes that contained large vacuoles and electron-dense bodies consistent with lysosomes were frequently found. Cellular debris intermingled with mercuric sulfide particles were scattered in the axoplasm (Figure 4A). Vacuoles and lipofuscin granules with mercury precipitation were also observed in the neuronal somas (Figure 4B). Macrophages that contained prominent mercury-laden lysosomes and lipofuscin granules were scattered in the superior cervical ganglion (Figure 5A). Macrophages that engulfed degenerating neuronal processes were sometimes found (Figure 5B).

## Discussion

We showed that intraneural injection of mercuric chloride produced significant toxicity in the peripheral nerve fibers. It cannot be suggested that intraneural injection of mercury compounds replicates the situation of systemic intoxication. Direct injection delivers mercury more rapidly and in higher concentrations than would be achieved by systemic administration. However, it should be pointed out that both direct injection and systemic administration of organic and inorganic mercury produce identical changes in the central nervous

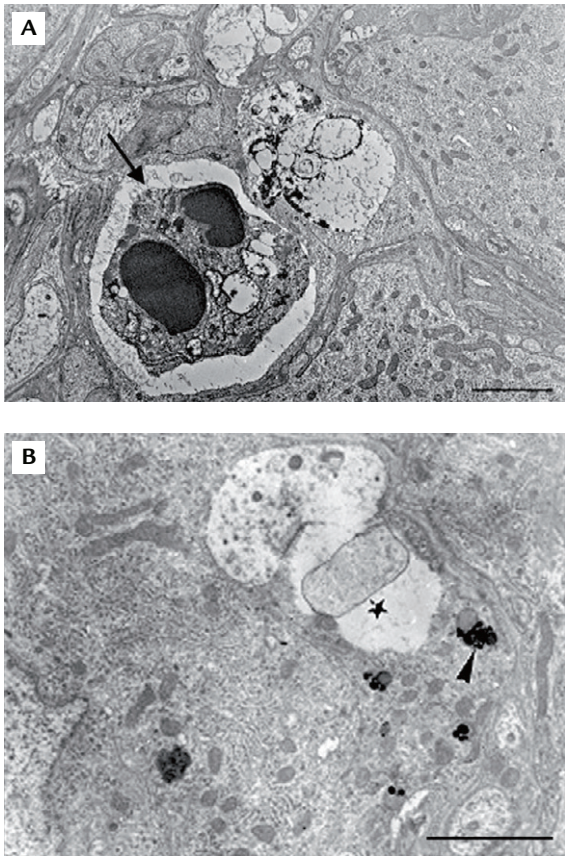


**Figure 4.** Ultrastructural changes in the superior cervical ganglion after intraneural injection of mercuric chloride. (A) Three days after injection, degenerating neuronal processes contained large vacuoles and electron-dense bodies intermingled with mercuric sulfide particles (arrow). Bar = 5.0 μm. (B) Vacuoles (asterisk) and lipofuscin granules with mercury precipitation (arrowhead) in a neuronal soma. Bar = 3.4 μm.

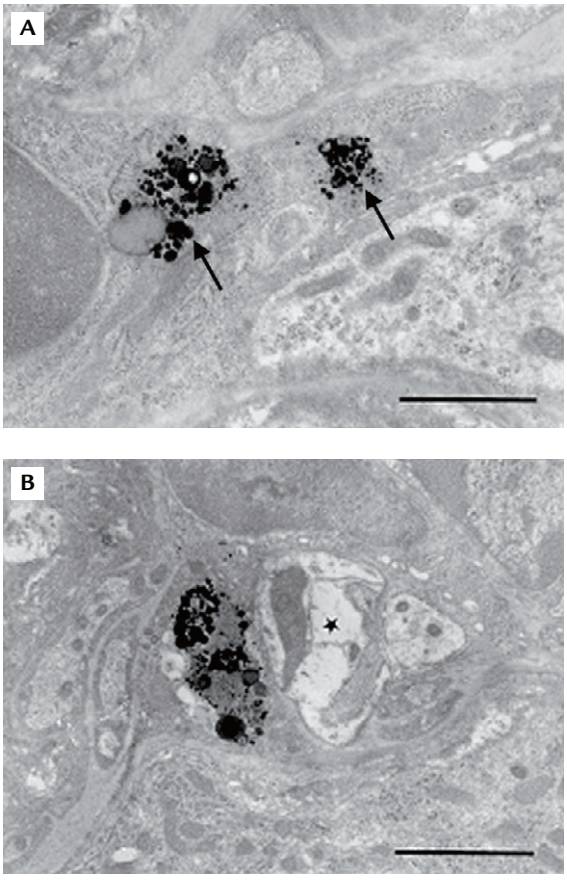


**Figure 5.** Phagocytic activity in the superior cervical ganglion after intraneural injection of mercuric chloride. (A) Macrophage contained prominent mercury-laden lysosomes and lipofuscin granules (arrows). Bar = 2.0 μm. (B) Macrophage engulfed degenerating neuronal processes (asterisk). Bar = 4.0 μm.

system[4] and peripheral nerves.[5] Direct injection has the advantage of minimizing the systemic influences of the toxin, and it is more certain that the changes result from the action of the toxin itself and are not secondary to damage of the vasculature or other organs. As shown in our study, a single injection of 150 μg mercuric chloride into the hypoglossal nerve resulted in no significant changes to the microvessels within the nervous system.

Using an autometallographic technique, retrograde transport of inorganic mercury has been demonstrated in axons of primary somatic motor and sensory neurons.[12] Although autometallography is a sensitive technique for metal detection, it can interfere with ultrastructural observations; and therefore, it was not used in our study. Instead, we used ammonium sulfide histochemistry to localize mercury in the nervous system and demonstrated accumulation of mercury in membrane-limited organelles within the nerve cell bodies in the superior cervical ganglion. As far as we know, this is the first report to describe uptake and retrograde transport of mercury by sympathetic axons.

Although mercury intoxication is associated with alterations in autonomic functions such as reduced heart rate variability,[9] direct effects of mercury on the autonomic nervous system have seldom been investigated. In the present study, we showed that intraneural injection of mercuric chloride into the hypoglossal nerve resulted in complete loss of unmyelinated postganglionic sympathetic fibers, whereas only minor changes were observed in the myelinated motor axons. A marked reduction in the number of unmyelinated axons has also been observed in rat sciatic nerves following intraneural injection of mercuric chloride.[5] However, the functional characteristics of unmyelinated fibers in the sciatic nerve are heterogeneous. By contrast, our findings clearly demonstrated that postganglionic sympathetic axons were unduly susceptible to mercury toxicity, as compared with somatic motor fibers. The selective chemical sympathectomy produced by mercuric chloride might have clinical applicability in situations in which blockade of sympathetic activity is beneficial, such as Raynaud's syndrome,[13] palmar hyperhidrosis,[14] and peripheral vascular disease.[15]

It is well known that peripheral sensory axons and cell bodies are more susceptible to mercury toxicity than their motor counterparts.[7,8] However, it is controversial whether the greater vulnerability is secondary to higher concentrations of mercury in the perikarya or to other functional attributes of sensory neurons.[16] In our study, we found that postganglionic sympathetic axons were more sensitive to mercury poisoning than were somatic motor fibers. Although the underlying reasons are not certain, this phenomenon could be explained, at least partially, by the presence or absence of myelin sheaths. Myelin sheaths and Schwann cells seemed to have protective roles. The changes in Schwann cells and the associated myelin were more obvious than those in the related axons. Moreover, fewer mercury particles were found in nerve cell bodies with myelinated axons than in those with unmyelinated axons. Besides the lack of myelination, the differences in protein repertoire, growth factor requirements, or electrical activities[16] are all possible contributing factors to the undue susceptibility of sympathetic fibers to mercury intoxication.

In conclusion, our study demonstrated uptake and retrograde transport of mercury by sympathetic axons, and mercuric chloride produced degeneration of postganglionic sympathetic nerve fibers, whereas somatic motor fibers were spared. The mechanisms that underlie the selective reaction of sympathetic fibers to mercury warrant further investigation.

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